

Social Interaction Ameliorates Stress-induced Worsening of Stroke Outcome in Mice

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By

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Abstract

Stress and social environment have been recently identified as important risk factors for stroke. Specifically, both stress and social isolation exacerbate stroke outcome through a mechanism that may involve the hypothalamic-pituitary-adrenal (HPA) axis. Conversely, affiliative social interactions have been shown to improve stroke outcome. The goal of the current study was two-fold: 1) to identify the mechanism by which social interactions modulate stroke outcome and 2) to determine whether social interactions provide a buffer against stress-induced exacerbation of stroke outcome. For this study, we used middle cerebral artery occlusion (MCAO) or SHAM surgery in mice to induce stroke. Male C57/bl6 mice were housed individually or paired with an ovariectomized female for two weeks prior to stroke and throughout the three day post-stroke recovery period. Mice in both housing conditions were assigned to stress (restraint) or non-stress conditions. Baseline and post-surgical behavioral testing was conducted on all animals. Our data indicate that following stroke, non-stressed, socially isolated mice showed a significant increase in infarct volume compared to pair-housed mice. However, among stressed mice, infarct volume did not differ by housing conditions. Analysis of behavioral testing indicated that following stress, socially isolated mice had significant functional deficits relative to all other groups tested. However, stressed pair-housed mice showed significant improvements in functional recovery and several behavioral measures after stroke indicating that post-stroke behavior among this group returned to baseline levels. The mechanism by which social interactions influence behavioral measures in the absence of differences in infarct volumes is currently under investigation. Taken together, these data show that 1) social interaction influences stroke outcome and 2) social interaction

buffers mice against stress-induced exacerbation of post-stroke functional deficits. We have begun to examine whether oxytocin, a peptide released during affiliative social interaction in several species, may underlie the neuroprotection provided by pair housing.

Introduction

Stroke remains the leading cause of adult disability and the third leading cause of death in developed countries (American Stroke Association, 2009). Stroke can affect many aspects of life, including loss of sensation and motor abilities; inability to speak properly or understand speech; changes in behavior, thought patterns, memories, and emotions (American Stroke Association, 2009). During an ischemic stroke, oxygen supply is cut off to regions of the brain via occlusion of a blood vessel in the brain. This results in cellular energy depletion by reducing the availability of adenosine triphosphate (ATP) in neurons, which in turn leads to an increase in intracellular Ca^{2+} due to pump failure and depolarization). This sets off a cascade of events that results in further neuronal injury during reperfusion, or the return of blood flow to the brain. Neuronal death following ischemia occurs by two mechanisms: necrosis and apoptosis. Necrosis is a passive process that occurs at the core of the ischemic lesion and is characterized by cellular swelling and lysis. The release of cellular contents from necrotic cells causes damage to neighboring cells and elicits an inflammatory response. Apoptosis is an active process that occurs at the periphery of the ischemic lesion. The inflammatory response that occurs in response to cerebral infarction causes a release of cytokines and other neurotoxic factors that may contribute to apoptotic neuronal death (White et al., 2000; Stoll et al., 1998).

Stress is a universal experience that results in activation of the hypothalamic-pituitary-adrenal (HPA) axis. HPA axis activity leads to sustained increases in circulating glucocorticoid concentrations (Sugo et al., 2002). Cortisol is the stress responsive glucocorticoid in humans whereas corticosterone (CORT) is released during stressful events in rodents. Glucocorticoids have many effects on the body including mobilization of energy stores for muscle use and

decreasing energy used for processes such as digestion and reproduction. Although increased HPA axis activity can be adaptive acutely, prolonged activation of the HPA axis can lead to numerous physical and mental pathologies. Stress and glucocorticoids are known for their ability to affect the immune system. In the early phase of stress before increased glucocorticoids have had a chance to affect target tissues, low concentrations of glucocorticoids stimulate immunity with the onset of stress. At higher concentrations, glucocorticoids begin to suppress the immune response. Glucocorticoids also exhibit a pro-inflammatory effect in some brain regions after neuronal insult and can lead to exacerbation of inflammation (Sorrells et al., 2007).

Stroke itself is a stressor. HPA activation is one of the first physiological responses to cerebral ischemia as measured by circulating glucocorticoids. Exposure to glucocorticoids peri-ischemia creates an environment in which neurons are less likely to survive subsequent injury (Sugo et al., 2002). Glucocorticoids inhibit glucose uptake in affected brain structures which potentiates neuronal ATP deprivation. As a result, affected neurons can no longer effectively pump cytosolic calcium, reuptake excitotoxic glutamate, or eliminate free oxygen radicals. This endangers affected neurons and may lead to neuronal death (Sorrells et al., 2007). Via the mechanisms described above, stress exacerbates stroke outcome. Stressed mice and rats have larger infarcts, behavioral deficits, higher neurological deficits, and a significant decline in cognitive function compared to their non-stressed cohorts (Sugo et al., 2002; Caso et al., 2007).

Although stress can have detrimental effects on stroke outcome, positive social interaction can be beneficial for recovery from stroke. Social interaction has been shown to influence recovery from disease and injury. In humans, individuals who are socially isolated, or

report lack of social support, have an increased incidence of myocardial infarction, recurrent stroke, and reduced survival following stroke compared to individuals with social support (Boden-Albala et al., 2005). Additionally, pair-housed male and female mice undergoing stroke had decreased infarct size compared to individually housed mice (Craft et al., 2005). Socially isolated mice have been shown to have increased infarct size, edema, and mortality compared to pair-housed mice undergoing stroke (Karelina et al., 2009). One proposed mechanism for this is social buffering of stress responses. Positive social interactions tend to dampen HPA axis activity while unpleasant social interactions tend to heighten HPA axis activity. As such, positive social interactions can provide a social buffer against stress. For example, positive social interaction in female Siberian hamsters improves wound healing through a mechanism that involves oxytocin-induced suppression of stress responses in the HPA axis (Detillion et al., 2004). These results suggest that social interaction improves stroke outcome, possibly through a mechanism that involves oxytocin-induced suppression of HPA axis responses to stress.

Oxytocin (OT) is a hormone that is synthesized in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the brain. Although it is best known for its peripheral role in lactation and parturition, it is also released centrally in response to positive social interaction and acts as a regulator of the HPA axis (DeVries, 2002). Recent research has begun to elucidate the anxiolytic role of OT. For example, when prairie voles, a socially monogamous rodent species, are introduced to a novel animal of the opposite sex, CORT concentrations decline rapidly in both sexes. A similar effect is also observed when prairie voles receive a single OT injection (DeVries, 2002). These results suggest that OT reduces anxiety and stress responses in prairie voles. In addition, the majority of studies on humans have indicated an anxiolytic effect

of OT. Data suggest that the HPA axis is suppressed in response to breast-feeding in both rats and humans, possibly due to the effects of OT release in the central nervous system (Heinrichs et al., 2002; Neumann et al., 2000). Another study indicates that intranasal administration of OT in humans enhances the buffering effect of social support against stress responsiveness (Heinrichs et al., 2003). These results suggest that OT plays an important role in the stress-protective effects associated with positive social interaction.

The goal for this study was to determine the effects of social interaction and stress on stroke outcome. The primary goal was to determine if social interaction acts as a buffer against stress and consequent effects on stroke outcome. We hypothesized that social interaction will improve stroke outcome and buffer against stress through OT-induced suppression of the HPA axis as measured by histological analysis and behavioral assays. We expected outcome to vary by housing condition and stress assignment. The group that we expected to exhibit the greatest behavioral deficits and neuronal damage was the socially isolated, stressed mice. We predicted that the socially isolated, unstressed mice would have significantly smaller infarcts and behavioral deficits than the socially isolated, stressed mice, but larger infarcts and greater behavioral deficits than all of the pair housed groups. We did not expect there to be significant differences between the unstressed, acutely stressed, and immediately stressed paired groups. To determine this, infarct development, functional recovery as defined by post-stroke behavioral measurements, circulating CORT, and central OT release were measured in paired and socially isolated mice.

Procedures and Methodology

Animals

Adult male and female C57/bl6 mice (Charles River; Wilmington, MA) were maintained in a temperature-controlled (~21°C) vivarium on a 14/10 hours light/dark cycle and allowed *ad libitum* access to food and water. Experimental male mice were housed either individually or with an ovariectomized female. The assigned housing conditions were maintained for 2 weeks before the study and throughout the 3-day reperfusion period. Animals were cared for in accordance with Institutional Animal Care and Use guidelines.

Surgery

Transient focal cerebral ischemia was induced in male mice by performing middle cerebral artery occlusion (MCAO). All surgeries were performed by authorized personnel as detailed in the approved animal use protocol. Briefly, unilateral MCAO was performed by inserting a 6-0 nylon monofilament into the internal carotid artery for 60 minutes. For SHAM operated animals, the internal carotid artery was exposed but not disrupted. Animals were randomly assigned to treatment groups. The treatment groups for this experiment and include (1) socially isolated, MCAO, acute stress (n=10); (2) pair-housed, MCAO, acute stress (n=10); (3) socially isolated, MCAO, no-stress (n=10); (4) pair-housed, MCAO, no-stress (n=10); (5) socially isolated, MCAO, immediate stress (n=9); (6) pair-housed, MCAO, immediate stress (n=9); (7) socially isolated, SHAM, acute stress (n=5); (8) pair-housed, SHAM, acute stress (n=5); (9) socially isolated, SHAM, no-stress (n=5); (10) pair-housed, SHAM, no-stress (n=5); (11) socially isolated, SHAM, immediate stress (n=6); (12) pair-housed, SHAM, immediate stress (n=3).

Restraint Stress

In this experiment, restraint stress was initiated either 22 hours (acute stress) or 2 hours (immediate stress) before surgery (See figure 1). Mice assigned to stress conditions were placed

in small Plexiglas tubes (3 cm internal diameter, 10 cm in length) for 2 hours. The restraint tubes are well ventilated and allow for minimal, confined movement but not head to tail turns.

Behavioral Testing

Baseline behavioral testing occurred 24 hours prior to surgery (See figure 1). The mice underwent behavioral testing again 3 days after surgery.

Paw Preference

Each mouse was placed in a vertical clear plastic cylinder (8 cm internal diameter, 12 cm in height) for 5 minutes while being videotaped. An experimenter recorded each time the mouse placed its paw or paws on the side of the cylinder, taking note of whether the right or left paw touched first. Paw preference was determined using the formula $[\text{left}/(\text{left}+\text{right}+\text{simultaneous})]\times 100$.

Open Field

Exploratory behavior was assessed in an open field apparatus using Flex Field photobeam activity (San Diego Instruments, San Diego, California). The apparatus consists of a clear Plexiglas insert (40 cm x 40 cm x 37.5cm) fitted inside a metal frame consisting of 16 equally spaced infrared photocell detectors. Interruptions in the infrared light sources by the experimental animal were recorded in the associated computer program. Data were analyzed for general locomotor activity and relative amount of activity occurring in the periphery versus the center of the apparatus (a 90cm² zone in the middle of the apparatus).

Determination of Stroke Volume

Mice were sacrificed by cervical dislocation according to the approved animal use protocol 3 days after surgery. Immediately after cervical dislocation and blood sampling, the

brains were removed and sectioned into five 2mm thick coronal sections. Sections were incubated for 10 minutes on each side in 2, 3, 5-triphenyltetrazolium maintained at 35°C and fixed in 10% formalin. The brain sections were then photographed and analyzed (Inquiry, Loats). The relative size of cortical infarct in each section is expressed as a percentage as follows: $100\% * [\text{contralateral cortex} - (\text{total ipsilateral cortex} - \text{cortical infarct})] / \text{ipsilateral cortex}$.

Histology

Histochemistry and immunohistochemistry were used to determine oxytocin expression within the PVN and SON. After infarct image analysis was completed, brain sections were stored in 10% formalin for approximately one week. The sections were then embedded in paraffin blocks, sectioned on a microtome at 5 µm, and mounted on slides. Briefly, slides were deparaffinized and heated in 10mM sodium citrate buffer for antigen retrieval. Tissue was then quenched with a 1% solution of hydrogen peroxide for 10 minutes and blocked in bovine serum albumin for 20 minutes. Rabbit anti-OT antibody was diluted 1:5000 in 0.1M PBS with 0.3% Triton-X100 and 2% normal goat serum. Tissue was incubated overnight at room temperature. The following day, tissue was incubated for 2 hours in biotinylated goat anti-rabbit antibody diluted 1:500 in 0.1M PBS with 0.3% Triton-X100 and 2% normal goat serum. Tissue was then immersed in Elite ABC in 0.1M PBS for 30 minutes. The reaction was then visualized using the DAB chromagen. Oxytocin was quantified using ImageJ (NIH) to measure staining density.

Determination of Blood Corticosterone Concentrations

Blood samples of 50 µl were collected after cervical dislocation and centrifuged at 4°C for 25 minutes at 13,000 rpm. Serum was then collected and stored at -80°C. CORT

concentrations were determined using ^{125}I radioimmunoassay kit (ICN Pharmaceuticals, Costa Mesa, CA, USA). All samples were analyzed in a single assay. All standard tubes were run in triplicate, and all unknown samples were run in duplicate.

Statistical Analysis

For statistical analysis, SPSS for Windows 17.0 (SPSS Inc., Chicago, IL.) was used. Behavioral data from the animals was analyzed via 3-way ANOVA (factors were housing, surgery, and stress). Infarct sizes and serum CORT concentrations were analyzed via ANOVA or independent samples t-test for pre-planned group comparisons.

Results

Infarct

The results of the ANOVA revealed that there was no main effect of housing ($F(1, 48) = 0.736$, $P > 0.05$) or stress ($F(2, 48) = 0.6$, $P > 0.05$) on infarct volume. However, the results did show an interaction between housing and stress ($F(2, 48) = 4.612$, $P < 0.05$). Using the independent samples t-test to compare the effects of acute stress and no stress on infarct, there is an effect of stress in both single housed ($t(16) = -1.752$, $P < 0.05$) and pair-housed mice ($t(17) = 2.308$, $P < 0.05$). Acutely stressed mice that are pair housed have increased infarct size while socially isolated mice have decreased infarct size. However, under no-stress conditions, socially isolated mice had significantly larger infarct size than paired mice. Immediately stressed animals showed no differences between pair-housed and socially isolated groups. See *figure 2*.

Paw Preference Behavior

Paw preference is a measure of behavioral lateralization typically observed following unilateral brain injury. The results of the 3-way ANOVA showed no effect of surgery on baseline paw preference (all $P > 0.05$) or post-surgical paw preference (all $P > 0.05$). However, the paired t-test showed a reduction in contralateral paw use in the paired immediate stress group after stroke ($t(6) = 2.918$, $P < 0.05$). *See figure 3.*

Open Field Behavior

Differences in baseline rearing activity, center activity, and total activity were all evaluated by 3-way ANOVA. Baseline rearing activity showed differences in housing ($F(1, 95) = 11.233$, $P < 0.05$) and stress ($F(2, 95) = 7.526$, $P < 0.05$). Following MCAO, there was a main effect of housing ($F(1, 40) = 6.094$, $P < 0.05$), and an interaction between housing and stress in post-stroke rearing activity ($F(1, 40) = 4.99$, $P < 0.05$). Rearing activity is a measure of exploratory behavior, and was decreased in all groups after surgery; however, socially isolated, stressed mice significantly decreased rearing activity relative to all other groups. Baseline center activity analysis revealed a main effect of stress ($F(2, 95) = 11.468$, $P < 0.05$). Following MCAO, there was a main effect of housing ($F(1, 40) = 4.729$, $P < 0.05$), as well as a housing by stress interaction: ($F(1, 40) = 4.291$, $P < 0.05$) on activity in the center. Time spent in the center of the open field is inversely proportional with anxiety-like behavior. All animals showed a decrease in the time spent in the center after surgery regardless of surgical group. However, socially isolated mice in the acutely stressed condition showed a significant decrease in time spent in center after MCAO relative to all other groups. There was also an effect of both housing ($F(1, 95) = 3.974$, $P < 0.05$) and stress ($F(2, 95) = 4.451$, $P < 0.05$) for baseline total activity. Following MCAO, there was a main effect of housing ($F(1, 40) = 4.204$, $P < 0.05$), as well

as a housing by stress interaction: ($F(1, 40) = 6.781, P < 0.05$) on total activity. Total activity is a measure of overall locomotor activity. All groups showed a decrease in locomotor activity after surgery ($F(1, 85) = 7.981, P < 0.05$). However, socially isolated, acutely and immediately stressed mice had the greatest decrease in locomotor activity post-stroke ($P < 0.05$) relative to all other groups. Socially isolated, acutely stressed mice exhibited the most substantial deficits between baseline and post-stroke behavior. *See figure 4.*

Serum CORT Concentrations

Results of the 3-way ANOVA indicate that there was an overall effect of housing ($F(1, 79) = 4.705, P < 0.05$) and surgery ($F(1, 79) = 37.774, P < 0.05$) on serum CORT concentrations. When comparing acutely stressed and non-stressed groups using an independent samples t-test, there is a difference in the single housed mice ($t(24) = 2.145, P < 0.05$). These results suggest that stroke alone is a stressor and the stress response to stroke can be further potentiated by social isolation and exposure to restraint stress. *See figure 5.*

Oxytocin

Changes in oxytocin immunoreactivity were analyzed in both the PVN and the SON by 3-way ANOVA. In the SON there were no effects of housing, stress, or surgery (all $P > 0.05$). In the PVN, mice undergoing immediate stress or no-stress showed no effects of housing or surgery (all $P > 0.05$). However, mice that underwent acute stress showed an effect of housing ($F(1, 21) = 4.721, P < 0.05$). Socially isolated, acutely stressed mice had overall greater OT immunoreactivity in the PVN than paired, acutely stressed mice. *See figure 6.*

Discussion

In this study, we examined the effects of stress and social interaction on stroke outcome. Ischemic damage and behavioral deficits were compared between socially isolated and paired mice undergoing no stress, acute stress, or immediate stress. In the non-stressed condition, socially isolated mice had increased infarct size relative to pair housed mice (*See figure 2*). This suggests that social interaction provides neuroprotection against ischemia in mice under non-stressful conditions. We examined central OT release in the PVN and SON as a possible mediator for the neuroprotection provided by social interaction. In the PVN, acutely stressed, socially isolated mice had increased central OT release in the PVN relative to paired mice (*See figure 6A*). Serum CORT concentrations were used as a measurement of the response to stress. Across all stress conditions, MCAO caused a significant increase in CORT relative to SHAM. Mice that had acute stress, MCAO, and were socially isolated had significantly greater CORT concentrations than all other groups (*See figure 5*). The open field test was used to assess functional outcome after surgery. The open field behavioral test results revealed that there were significant increases in anxiety and decreases in overall locomotor activity and exploratory behavior after surgery in all groups, but the most significant differences were observed in the acutely stressed, socially isolated, MCAO mice (*See figure 4*). These results, taken together, suggest that even in the absence of increased infarct size, acutely stressed, socially isolated mice suffer from severe functional deficits post-stroke.

The acutely stressed animals experienced the most drastic decreases in functional outcome even in the absence of increased infarct volume. A previous study found that mice housed with several other mice in standard cages as opposed to environmentally enriched cages after stroke had decreased motor function despite a lack of differences in infarct volume

(Nygren et al., 2005). In this study, preconditioning to a stressful stimulus in the single, acutely stressed, MCAO animals may account for the nonsignificant trend for decreased infarct volume relative to non-stressed animals (*See figure 2*). Preconditioning refers to exposure to a noxious stimulus near to or below the threshold of damage resulting in immediate or delayed tolerance of similar stimuli beyond the threshold of damage (Dirnagl et al., 2009). Indeed, various sublethal stressors such as brief periods of ischemia, low doses of inflammatory stimuli, and short applications of anesthesia can be neuroprotective in future ischemic events (reviewed in Dirnagl et al., 2009). The 2 hours of restraint stress experienced 22 hours before surgery may have pre-conditioned the acutely stressed animals to the stressful stimulus of a stroke. There was a trend for increased infarct volume in paired animals exposed to stress relative to non-stressed paired animals (*See figure 2*). Although preconditioning may have prevented neuronal death in the single, acutely stressed mice, the damage to the neurons is significant enough to cause common behavioral deficits following stroke to still develop. Socially isolated, acutely stressed, MCAO mice had increased CORT concentrations and decreased functional outcome relative to all other groups. This suggests that the stress of stroke is potentiated by acute stress and worsens post-stroke outcome. Although the mechanism is not understood, it is clear that immediate stress did not affect behavioral outcome or infarct volume to the same extent that acute stress did in this study. Instead, both paired and single mice in this group had large infarct volumes. This is likely because the immediate stressor produced a ceiling effect resulting in exacerbated infarct regardless of housing condition. Because socially isolated, acutely stressed animals had greater functional deficits than their paired cohorts, it is reasonable to suggest that social interaction provides a buffer against stress in this stroke model.

Social interaction has been shown to buffer against stress and improve disease outcome in both humans and rodents. In humans, individuals who are socially isolated prior to stroke exhibit significantly greater decline in stroke outcome in the following 5 years (Boden-Albala et al., 2005). In mice, animals that were paired had significantly decreased infarct size and improved contralateral paw use relative to socially isolated cohorts (Craft et al., 2005). This study further elucidated the effects of pairing on stroke outcome in mice by showing that pairing rescued mice from the harmful effects of stress on stroke outcome. Paired, acutely stressed, MCAO mice had a much improved outcome post stroke relative to their socially isolated cohorts. Although the mechanism is still not understood, simply having positive social contact with an ovariectomized female provides protection from stress.

In this study, the socially isolated, acutely stressed, MCAO mice which had the greatest anxiety and most severe functional deficits also had the greatest serum CORT concentrations (*See figure 5*). In mice, exposure to chronic social stress or exogenous CORT prior to stroke increases infarct volume and exacerbates cognitive deficits. However, treatment with a glucocorticoid receptor antagonist before stroke ameliorates these effects of stress and improves stroke outcome (Sugo et al., 2002). This suggests that the elevated CORT levels may be the cause for increased functional deficits in socially isolated, acutely stressed, MCAO mice. To test this hypothesis, we are now conducting a study in which all previously described procedures remain the same, but the mice are being injected with either a glucocorticoid synthesis inhibitor or saline prior to baseline behavior, before stroke, and daily throughout the 3-day reperfusion period. If CORT is indeed underlying the harmful effects of stress on stroke

outcome, we expect the animals treated with the glucocorticoid synthesis inhibitor to have reduced functional deficits compared to saline treated mice.

This study provides evidence that social support provides a buffer against stress and improves stroke outcome. One possible mechanism for the neuroprotection provided by pairing is central OT release. We expected OT to be increased in paired animals relative to socially isolated animals because OT is released in response to positive social interactions. In the PVN, acutely stressed, socially isolated mice had significantly greater OT relative to acutely stressed, paired mice (*See figure 6A*). This could be because OT is released in response to stress, as well as social contact. Increased OT release during stress may be a coping mechanism for the animal, decreasing stress-induced anxiety. For example, studies have shown increased OT release in response to restraint stress, ether, hypoglycemia, and osmotic stress in rats (Jezova et al., 1995). Although this study did not provide any conclusive data that OT mediates social buffering, it is still a possible mediator and requires further investigation. One possible explanation for a lack of conclusive data is that OT may have already been released into the periphery in the paired animals, but not the socially isolated animals at the time we examined central OT. Some other techniques that could be used to assess OT are analyzing the activity of OT receptors within the brain and measuring circulating OT levels.

The results of this study show that although stress exacerbates stroke outcome, positive social interaction can buffer against these effects. Clinically, this implies that recovery from stroke in humans, particularly in stressed individuals, may benefit from incorporating positive social stimuli such as support groups or therapy. Although pairing did not decrease infarct volume in acutely stressed mice, it improved functional recovery. Functional recovery from

stroke is arguably more relevant to stroke survivors than size of their infarct indicating that this study has important potential clinical implications. In this respect, simple contact and interaction with friends and family may improve functional recovery from stroke in humans. Future studies should focus on identifying the mechanism through which social interaction buffers against stress. Follow up studies should also examine if administering a glucocorticoid antagonist will eliminate the increased CORT and extreme functional deficits witnessed in the socially isolated, acutely stressed, MCAO mice.

Figure 1

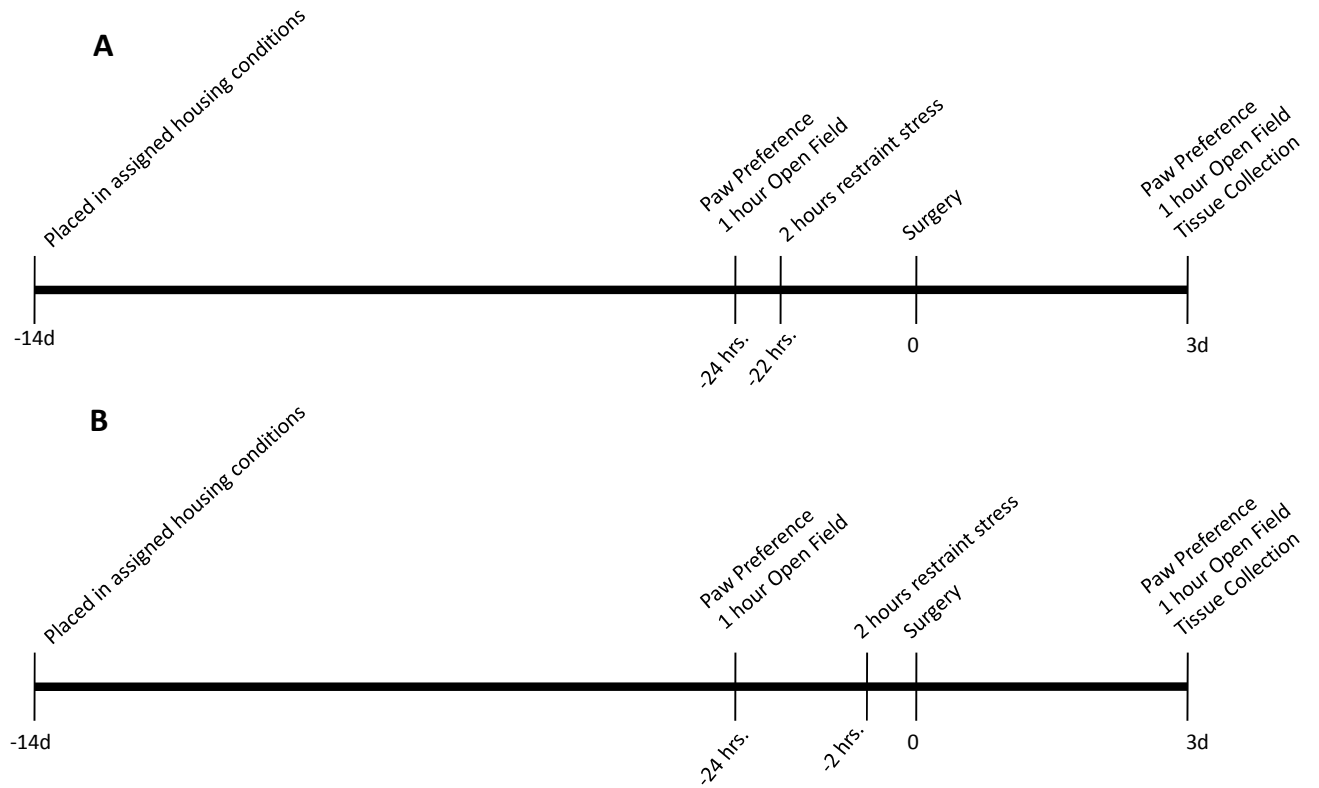


Figure 1. Timeline of experimental events in A) acutely stressed mice and B) immediately stressed mice. Non-stressed mice underwent the same schedule excluding 2 hours of restraint stress.

Figure 2

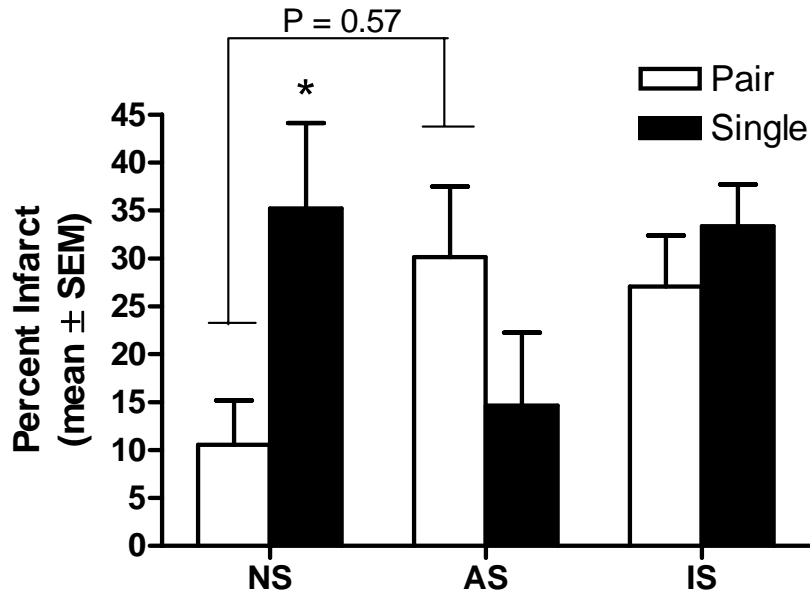


Figure 2. Infarct volume is a measure of ischemic damage. Housing did not affect infarct volume in acute stress (AS) or immediate stress (IS) groups. In the non-stressed (NS) group, socially isolated mice had significantly increased infarct volumes relative to paired mice. There was also a trend in paired mice for increased infarct volume in acutely stressed mice relative to non-stressed.

Figure 3

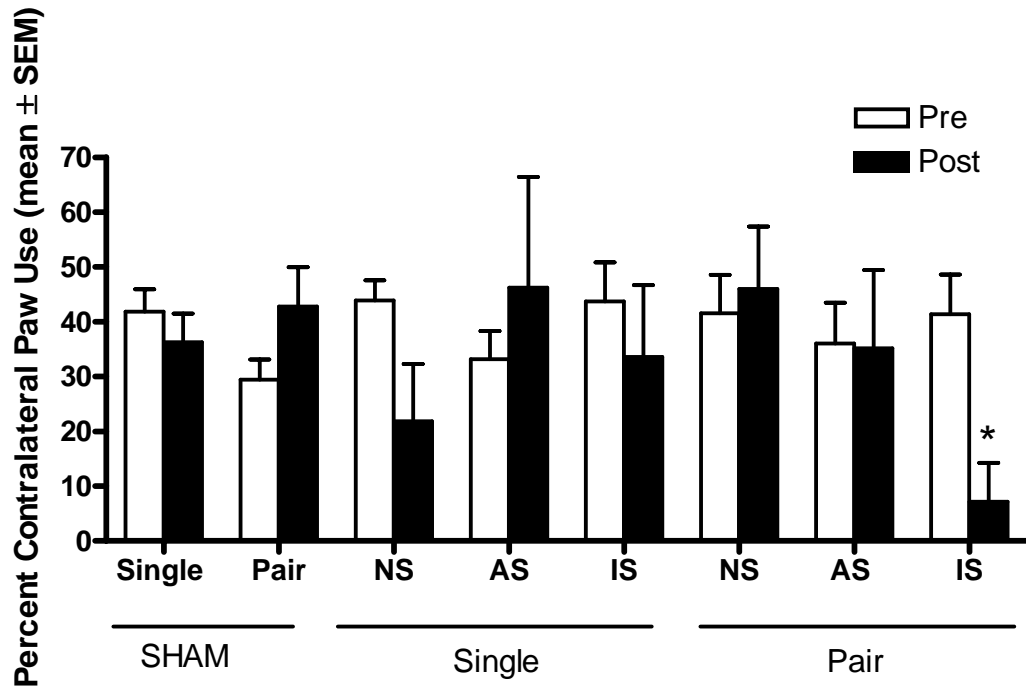


Figure 3. Paw Preference. Paired, immediately stressed, MCAO mice had significantly decreased contralateral paw use after stroke. NS= non-stressed, AS= acutely stressed, IS= immediately stressed.

Figure 4

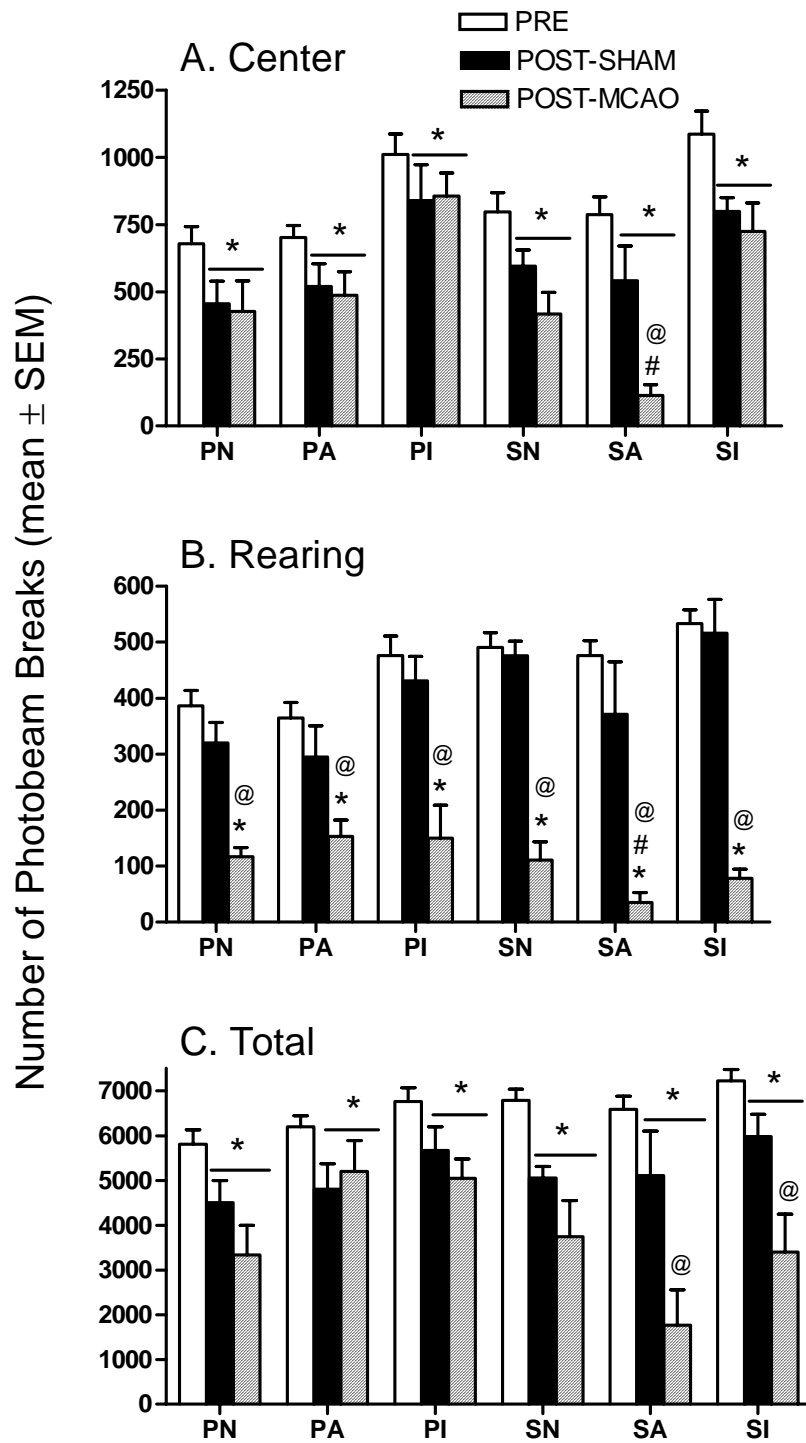


Figure 4. Open Field Behavior. A) The number of beam breaks in the center is inversely proportional to anxiety-like behavior. Paired, non-stressed (PN), paired, acutely stressed (PA), paired, immediately stressed (PI), single, non-stressed (SN), single, acutely stressed (SA), and single, immediately stressed (SI) animals all exhibited a decrease in the number of beam breaks after surgery independent of surgical group. However, SN and SA showed a stepwise decrease in the number of beam breaks in the center with SA mice showing the greatest decrease from baseline behavior. B) Rearing is a reflection of exploratory behavior. All groups showed a decrease from baseline behavior. MCAO animals in all groups had significantly decreased rearing relative to SHAMs after surgery. However, SA mice showed the greatest decrease in rearing from baseline behavior. C) Total activity is a measure of overall locomotor activity. All groups decreased total activity significantly after surgery. SA and SI had significantly decreased total activity relative to SHAMs in both groups. These data indicate that paired mice are buffered against stress-induced post-stroke functional deficits. @= different from SHAM; #= different from paired, acutely stressed.

Figure 5

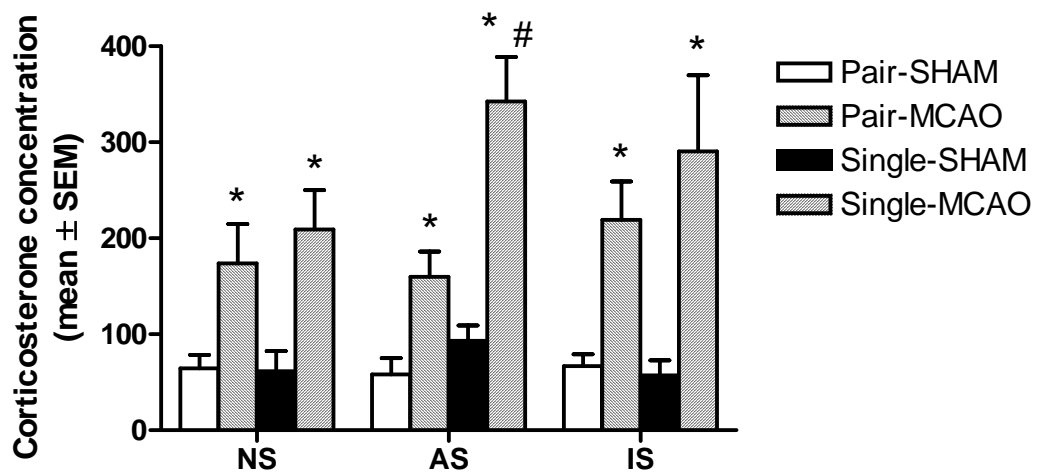


Figure 5. Serum CORT concentrations after the 3-day reperfusion period. MCAO significantly increased CORT in MCAO mice independent of stress and housing. Socially isolated, acutely stressed, MCAO mice had significantly increased CORT concentrations relative to paired, acutely stressed, MCAO mice (#). These data indicate that paired animals were buffered against further increase in CORT following restraint stress.

Figure 6

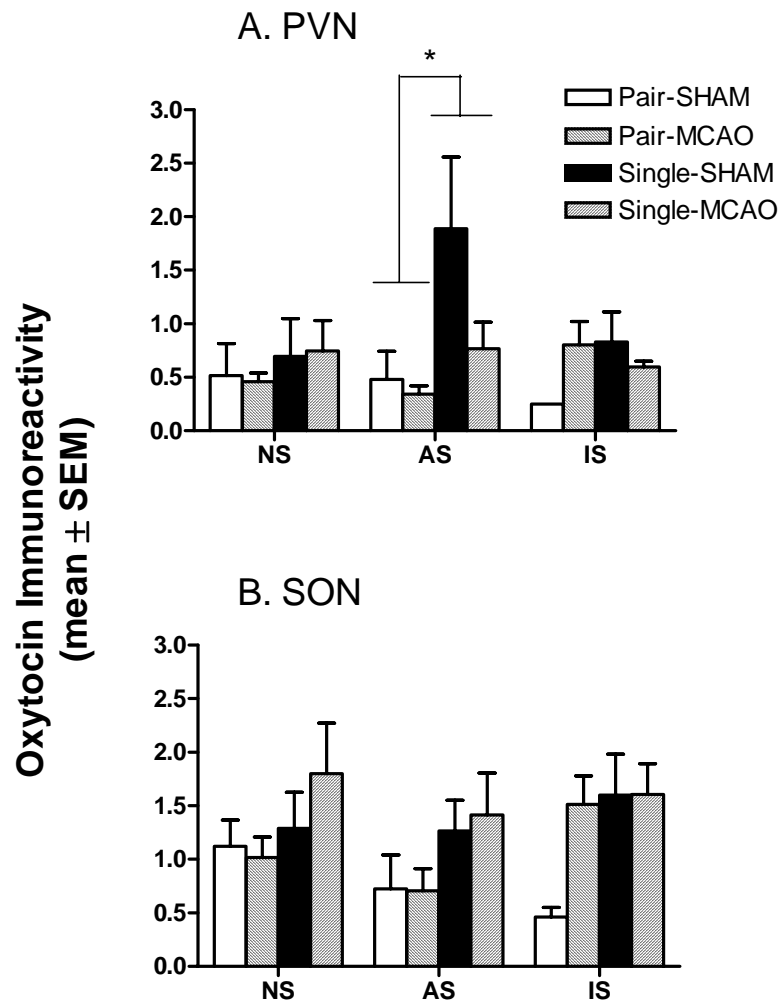


Figure 6. Oxytocin Immunoreactivity in A) the paraventricular nucleus (PVN) and B) the supraoptic nucleus (SON). In the PVN, socially isolated mice in the acutely stressed condition have increased central oxytocin release relative to their paired cohorts. NS= non-stressed, AS= acutely stressed, IS= immediately stressed.

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